PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	24 MAR 2006
Applicant's or agent's file reference NEB-238-PCT	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US05/02029	21 January 2005 (21.01.2005)	23 January 2004 (23.01.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC(8): C12Q 1/68; A01N 43/04; C07H 21/04; A61K 31/07 and US Cl.: 435/6, 91.1, 325, 375; 536/23.1, 24.3, 24.33, 24.5; 514/44

Applicant

NEW ENGLANG BIOLABS, INC.

1. This opinion contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	04 January 2006 (04.01.2006)	Terra C. Gibbs Telephone No. 571-272-0564

Form PCT/ISA/237 (cover sheet) (April 2005)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☒ on paper

☒ in electronic form

c. time of filing/furnishing

☒ contained in the international application as filed.

☒ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 3, 4, 10, 13, 14, 16-23, 25, 29, and 30	YES
	Claims 1, 2, 5-9, 11, 12, 15, 24, and 26-28	NO
Inventive step (IS)	Claims 3, 4, 10, 13, 14, 16-23, 25, 29, and 30	YES
	Claims 1, 2, 5-9, 11, 12, 15, 24, and 26-28	NO
Industrial applicability (IA)	Claims 1-30	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-30 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 3, 4, and 10 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of preparing a hsiRNA mixture comprising reacting a preparation of double-stranded with a mutant RNase III, wherein the mutant RNase III has a mutation in the position corresponding to E38 or E65 in *E. Coli* RNase III.

Claims 13, 29, and 30 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of down-regulating gene expression of a target gene comprising preparing a heterogenous siRNA mixture using a mutant RNase III, causing fragments from the siRNA mixture to degrade mRNA, and down-regulating gene expression, wherein the mutant RNase III has a mutation in the position corresponding to E38 or E65 in *E. Coli* RNase III.

Claims 14, 16-23, and 25 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of down-regulating gene expression of a target gene comprising preparing a heterogenous siRNA mixture using a mutant RNase III, causing fragments from the siRNA mixture to degrade mRNA, and down-regulating gene expression *in vivo*.

Claims 1, 2, 5-9, 11, 12, 15, 24, and 26-28 lack an inventive step under PCT Article 33(3) as being obvious over Byrom et al. in view of Sun et al. Byrom et al. teach inducing RNAi with siRNA cocktails generated by RNase III. Sun et al. teach the importance of certain residues within the RNase III protein for biological activity. It would have been obvious to one of ordinary skill in the art to cleave dsRNA with RNase III to make siRNA for the purpose of inhibiting gene expression via RNA interference as taught by Byrom et al. One of ordinary skill in the art would have been motivated to use a mutant RNase III since Sun et al. taught that specific RNase III mutants exhibit enhanced catalytic activity. Therefore, the invention would have been prima facie obvious to one of ordinary skill in the art at the time of filing.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Please See Continuation Sheet

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

VIII. The following observations on the clarity of the claims, description, and drawings or on the questions, are made:

Claims 14, 16-23, and 25 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. The description does not disclose the claimed invention in a manner sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because: the disclosure does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention.

The amount of guidance, direction, and exemplification set forth in the disclosure would not be sufficient to enable the skilled artisan to use the claimed invention, to down-regulate gene expression of a target gene *in vivo*, comprising administering a siRNA generated from a dsRNA cleaved with a mutant RNase III, without a need to first perform an undue amount of additional experimentation. The following factors have been considered in formulating this rejection: the breadth of the claims, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, the amount of direction or guidance presented, the presence or absence of working examples of the invention, and the quantity of experimentation necessary.

The instant claims are drawn to a method of down-regulating gene expression of a target gene *in vivo*, comprising administering a siRNA generated from a dsRNA cleaved with a mutant RNase III.

The disclosure as filed contemplates and claims the administered siRNA provides a treatment for a disease in a mammal (see claim 18). However, there are no examples wherein a siRNA is administered to a mammal or used to treat any disease in a mammal. At the time the instant Application was filed, and even to date, the field of RNA interference was in its infancy and gene specific dsRNA inhibition in mammalian cells was highly unpredictable. Even with the advances made by the field of RNA interference, including inducing inhibition by RNA interference in mammalian cells in culture, RNA interference is recognized in the art as not enabled for therapeutic purposes (see for example, Caplen et al., Gene Therapy, 2003 Vol. 3:575-586, Coburn et al., J Anti Chemo, 2003 Vol. 51:753-756, and Agami et al. Curr Opin in Chem Bio, 2002 Vol. 6:829-834 for a review on the progression of RNA interference in mammalian cells and the state of the art of RNA interference for therapeutic purposes).

For example, Caplen et al. points out, "Many of the problems associated with developing RNAi as an effective therapeutic are the same as encountered with previous gene therapy approaches. The key issues of delivering nucleic acids to the required cell type, while ensuring an appropriate level of efficacy with minimum toxicity induced by the vector system have been problems the gene therapy field has struggled with for over a decade now" (see page 581, first and second columns).

Coburn et al. also points out that the major impediment to using RNA interference as a therapeutic is that gene expression is transient and the delivery methods used for RNAi are not effective for therapeutic purposes (see for example page 54, first column, last paragraph).

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

Agami et al. teach, "As it stands, the application of siRNAs for disease and gene therapies can follow the existing tools that are already applicable for clinical trials of siRNA strategies to inhibit gene expression. However, a major drawback of this technology is its transient effect" (see page 832, second column).

The field of RNA interference is optimistic about the potential of RNA interference as a therapeutic tool, but even with the advances made subsequent to the filing of the instant Application, the field recognizes that therapeutic methods are not yet effective.

Given this unpredictability, the skilled artisan would require specific guidance to practice the claimed methods *in vivo*, with a resultant therapeutic outcome, as claimed. The field of siRNA, to date, does not provide guidelines by which siRNA can be routinely delivered to generally any cell type *in vivo* (whole organism) at a concentration effective to result in a therapeutic effect. The disclosure does not provide specific guidance by which one skilled in the art would expect to be able to deliver an siRNA to generally any target cell or tissue *in vivo* (whole organism) at a concentration effective to treat a disease in a mammal as encompassed by the claims.

In order to practice the invention claimed, one skilled in the art would need to undergo undue trial and error experimentation, beyond the teachings of the instant disclosure. The quantity of undue experimentation would include the determination of how to effectively target and deliver an effective concentration of siRNA to specific cells to a target cell *in vivo* to achieve a treatment effect. Additionally, this undue experimentation would include the determination of such factors as dosage, route of administration, disposition of the siRNA in tissues, and the half-life and stability of the siRNA molecule *in vivo*. Given the art recognized unpredictability of the therapeutic application of siRNA *in vivo* (whole organism), this determination would not be routine and would require undue trial and error experimentation.

Therefore, due to the broad scope of the methods claimed, the state of the art of siRNA therapy, the level of unpredictability of *in vivo* (whole organism) methods of using siRNA, the lack of specific guidance for the *in vivo* (whole organism) application of siRNA methods for therapeutic benefit, and the lack of working examples or examples which correlate with the claimed methods, one skilled in the art would not be able to practice the methods without undue trial and error experimentation.